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## ORIGINAL ARTICLES

**Cloning of the gene encoding the 1-aminocyclopropane-1-carboxylate (ACC) deaminase to E. coli and study of its expression**Sadrnia M.<sup>1</sup>, Maksimava N.<sup>1</sup>, Khromsova E.<sup>1</sup>, Stanislavich S.<sup>1</sup>, Owlia P.<sup>2,3</sup>, Arjomandzadegan M.<sup>4</sup><sup>1</sup> Department of Genetics, Faculty of Biology, Belarusian State University, Minsk, Belarus;<sup>2</sup> Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran;<sup>3</sup> Antimicrobial Resistance Research Center, Tehran University of Medical Sciences, Tehran, Iran;<sup>4</sup> Tuberculosis and Pediatric Infectious Research Center, Arak University of Medical Sciences, Arak, Iran

**Aim.** The purpose of the study was to optimize the conditions for transferring the aminocyclopropane-1-carboxylate (ACC) gene (isolated from a rhizosphere bacterium) to *Escherichia coli*.

**Methods.** A collection of 18 rhizosphere bacteria of the genus *Pseudomonas* was screened for the presence of the *acdS* gene by PCR and ACC deaminase activity assay. Purified acds was ligated on plasmid pXcmK12 to yield a pACC3 plasmid carrying the *acdS* gene. The recombinant plasmid pACC3 was transferred to *Escherichia coli* DH5 alpha (*E. coli* DH5α) cells. Restriction analysis of the plasmid was accomplished at the Bam HI and SmaI restriction sites to confirm cloning of the gene. Enzyme production assay was done to ensure gene expression in transformed *E. coli* DH5α, in comparison with *E. coli* DH5α (as control) and *P. mendocina* BKMB 1299.

**Results.** Three out of eighteen strains of *Pseudomonas* harbored the gene encoding ACC deaminase. The highest specific ACC deaminase activity was detected in *P. mendocina* BKMB 1299. The successfully purified fragment was ligated in the T-vector and cloned in *E. coli* DH5α cells. The transformed cells grown in culture medium containing antibiotics demonstrated plasmid transmission. pACC3 plasmid (recombinant) purified from the cells was successfully restricted at Bam HI and SmaI, confirming that the *acdS*-gene was cloned in the bacterium. Comparison studies of measured enzyme production (gene expression) by alpha-keto butyrate production assay in transformed *E. coli* DH5α, *E. coli* DH5α, and *P. mendocina* BKMB 1299 revealed the formation of 0.0, 0.4 and 1.06 μmol alpha-ketobutyrate mg protein/min, respectively.

**Conclusion.** The gene encoding ACC deaminase was transferred to *E. coli* DH5α and successfully expressed. In the next step, the gene should be cloned in proper rhizosphere bacterium.

*lingua: Inglese***FULL TEXT****ESTRATTI**

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